Developing New Drugs and Personalized Medical Treatment

Livermore research is behind a new technique for precisely measuring how drugs affect people.

Livermore built the first accelerator mass spectrometer specifically designed for biological research. Physicist Ted Ognibene was a key member of the design team.

IMAGINE attaching a bit of carbon-14 to a minute quantity of an antibiotic being developed to treat patients exposed to a biothreat agent or an emerging disease. The extremely small drug dose is nontoxic, and the amount of radiation is nearly negligible. This "microdose" is given to a small group of healthy people to determine whether the drug finds its way to the sites in the body that are thought to harbor infection. Blood and tissue samples taken from the subjects over the following hours, days, or weeks are run through an accelerator mass spectrometer, which can measure the amount of carbon-14 and, therefore, the amount of the drug in the body. These analyses reveal not only how much of the new drug is distributed to targeted sites but also how long it resides in the body. These data allow researchers to determine an optimal dosage that could counteract infection while minimizing side effects. A significant benefit to this so-called microdosing technique is that expensive and time-consuming animal testing would not be required. Microdosing studies on humans would provide a faster, more cost-effective way to deploy new countermeasure drugs. In the next year, Laboratory researchers hope to apply this method to new drugs that counter biological and radiological threats.

In a similar scenario, a microdose of an established drug tagged with carbon-14

is given to a patient sick with a difficult-to-treat infection. Tests taken just a few hours later would measure the amount of the drug in the body and its location. The resulting data could allow the physician to effectively adjust the standard dosage for that individual, minimizing side effects and increasing the patient's chances for a full recovery. Lawrence Livermore is working with oncologists to test this method on cancer patients and volunteer subjects.

The accelerator mass spectrometer is the essential tool that makes these scenarios possible. The amounts of the drugs and carbon-14 being measured are so small that no other instrument can detect the miniscule quantities, much less measure them.

Highly sensitive accelerator mass spectrometry (AMS) has most often been used for carbon-14 dating in research areas such as archaeology, paleoclimatology, and paleobotany. (See the box on p. 18.) Livermore researchers were the first to apply AMS to biological research almost 20 years ago. The earliest biological experiment at the Laboratory's Center for Accelerator Mass Spectrometry (CAMS) examined the effects of low doses of a suspected carcinogen on mouse DNA. Today, AMS studies of new drugs, nutrients, and toxic compounds can use human subjects because the safety of AMS has been demonstrated repeatedly. The amount of radioactive carbon-14 used to tag a biomolecule is less than the naturally occurring cosmic radiation an airline traveler encounters during a routine flight. The carbon-14 moves through the body without disturbing normal metabolic processes, even when it remains for many days or weeks.

Some human subjects for AMS studies are healthy volunteers and others are patients. Doctors and researchers at the University of California (UC) Davis Cancer Center have collaborated with Livermore scientists for more than a decade in such studies. Particular patients volunteer to help researchers learn more about the effects of commonly used cancer chemotherapy drugs. Doctors know that drugs such as carboplatin are highly effective at destroying cancer cells. Interestingly, researchers found that this drug works well for treating testicular cancer, but the results for other types of cancer have been much less spectacular. Why is this the case?

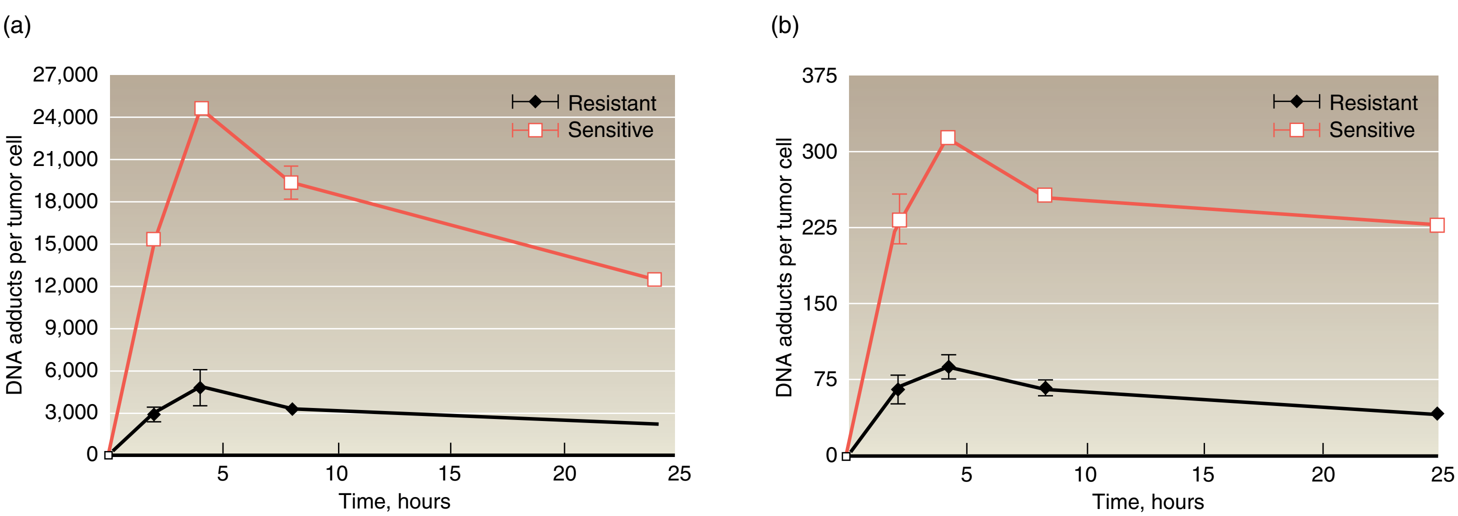
The specifics of a drug's absorption, distribution, metabolism, and excretion (ADME)—its pharmacokinetics—are poorly understood, and better information is needed to provide the answer. AMS, the most effective tool for human pharmacokinetic studies, will be a boon to drug companies for ADME research on new drugs.

Accelerating Drug Development

ADME studies performed early in the development of a new drug could be a deciding factor in whether a pharmaceutical company will continue its pursuit to market the drug. "Ninety percent of the drugs that start on the path to market don't cross the finish line," says biochemist Ken Turteltaub, a codeveloper of AMS for biological research. Developing a new drug typically takes 10 to 12 years and can cost as much as $1.5 billion. Making the "go" or "no-go" decision as early as possible could save pharmaceutical companies—and consumers—billions of dollars.

Drug development often requires 6 to 8 years of laboratory research, which is then followed by injecting laboratory animals with the drug and extrapolating the measured effects to a human-relevant dose. If the animal test results seem promising, Phase I clinical trials follow. Phase I testing typically involves 10 to 20 healthy human subjects who volunteer to participate. They are given the new drug at clinical doses to evaluate its safety, determine a safe dosage range, and help identify its possible side effects. Subsequent Phase II and III trials involve a larger number of human subjects, who are patients diagnosed with the specific illness that scientists hope the drug will help treat or cure.

The process often fails at the point where Phase I clinical trials begin. Research has repeatedly shown that humans and animals metabolize many substances differently. For example, chocolate is poisonous to a dog but can be a delicious treat for a child of similar weight because humans metabolize the theobromine in chocolate much more quickly than dogs. A decade ago, Turteltaub and colleagues compared the effects of PhIP, a substance that forms in meats during cooking, on humans and rodents. PhIP damages DNA and is carcinogenic to rodents at high doses, causing colon, breast, and prostate tumors. The team found that DNA damage in the colon was 5 to 10 times higher in humans than in rats given comparable doses based



Some chemotherapy drugs form damage products, called DNA adducts, that are toxic to rapidly dividing cells. Carboplatin is one such drug. Some patients are "sensitive" to carboplatin, while others are "resistant" to the drug (that is, fewer adducts are created). Laboratory tests show (a) cellular response to a full dose of carboplatin that a patient would receive during chemotherapy and (b) cellular response to a dose 1/100th of that amount, called a microdose. The two responses are similar, indicating that experiments using microdoses of carboplatin could be useful for predicting the pharmacokinetics of this drug in humans. (Courtesy of Chong-xian Pan and Tao Li of the University of California at Davis.)

on body weight. The consensus in this case was that humans must also metabolize PhIP differently.

Microdosing can facilitate the leap from animal to human studies. In a microdose study, a person receives just 1/100th of the clinical dose of a drug. This dose contains a small amount of the isotope carbon-14, just enough for the drug's ADME to be measured using AMS. The tiny doses are sufficient to study cellular response but not large enough to produce either therapeutic or toxic effects in the individual. Because of the sensitivity of AMS, only very small samples of blood, urine, biopsy tissue, or cerebrospinal fluid are needed for the tests that follow.

"Tests to date comparing microdoses and clinical doses of particular drugs indicate that the cellular responses are similar," says Turteltaub. "Only a small number of drugs has been tested so far, and much more work needs to be done to validate these findings. Microdosing may not work for all compounds."

If microdosing does prove to be an effective tool, it will help researchers determine if a candidate drug has the properties needed to reach targeted tissues and then remain at sufficient levels for a specified period. Researchers could more quickly determine which compounds have the proper pharmacokinetic properties and eliminate those that do not, allowing more time and money to be focused on the most promising candidates. Drugs could reach the marketplace faster, and their development would be more cost effective. These early tests could also give developers information on which individuals are likely to benefit from the drug—the foundation of personalized medicine.

In addition to moving new drugs more quickly to the marketplace and into the hands of health providers, AMS tests might help researchers better understand the interactive effects when more than one drug is injected. AMS and microdosing could allow for studying the interactions of multiple drugs because the effects of such tests would be so small. Frequent sampling of human subjects could provide highly accurate data about drug behavior and metabolism, resulting in improved models. Three private companies have licensed Livermore's AMS biomedical technology with the goal of managing microdosing studies for pharmaceutical firms developing new drugs.

Some accelerator mass spectrometry measurements require that samples be converted to small cylinders of graphite before they are ionized inside the spectrometer. Michale Kashgarian places the sample holder.

Less Expensive AMS

Two developments are behind the increased use of AMS: smaller, less expensive accelerators and faster, less labor-intensive sample processing. Livermore's primary AMS system, in place since 1989, is the size of a basketball court. Ten years ago, the Laboratory custom-built the first accelerator mass spectrometer dedicated to carbon-14 biological research. This 1-megavolt machine is just one-tenth the size of the larger spectrometer.

Physicist Ted Ognibene, whose specialty is AMS instrumentation, says, "AMS researchers have since discovered that even 1 megavolt is more energy than necessary. Half that energy, or 500 kilovolts, is plenty. Since 2002, a U.S. firm has been building 500-kilovolt systems at a cost of $1 to $2 million." An even smaller machine is being tested that uses just 200 kilovolts. However, according to Ognibene, the jury is still out on that one. He says, "Most of these small systems are for natural carbon. They would work for biological research, but few scientists are using them in this way."

Livermore's 1-megavolt spectrometer serves as a test bed for new sample preparation and delivery technologies. Now, some samples containing carbon-14 are run through a high-performance liquid chromatograph (HPLC), which separates the compounds and creates mini-samples called fractions every 15 seconds or so. Carbon is added, and the fractions are converted to small cylinders of graphite. The samples are placed in the ion source, where they are ionized. Then they travel through the accelerator into the spectrometer, where the ionized particles are counted. "It takes about a day to prepare 60 individual samples and 4 hours to run them through the AMS machine," says Ognibene. "Each sample costs about $150 to produce." Multiply that cost and time by the days and weeks of a drug test, and the roadblock that has kept AMS from broader, routine use becomes obvious.

Ognibene and others at Livermore are working to change that daunting arithmetic. They are developing methods whereby carbon-14 can be measured directly from HPLC, avoiding graphite sample preparation. Continuous-flow HPLC AMS, "a name that is admittedly a mouthful," notes Ognibene, "is still a few years away. Several interfaces have to work perfectly."

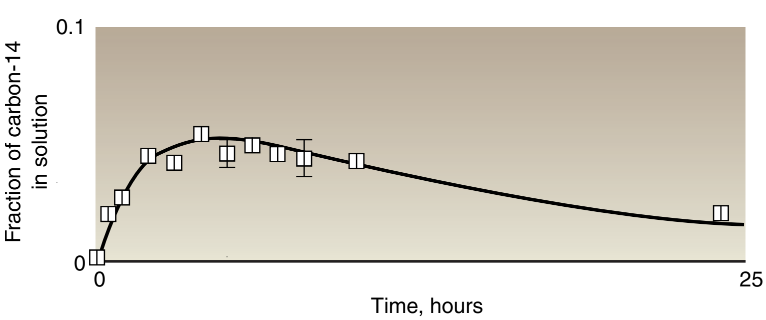
Output from HPLC will connect to a combustion chamber that breaks down the sample material and sends it directly to the AMS ion source. In 2007, the team installed a new ion source that can accept sample material in gaseous form. The next step, which will take another year, is to align the HPLC with the spectrometer.

The current system of collecting fractions every 15 seconds or so generates data at those same intervals. The continuous-flow process will offer better ADME data because the spectrometer can make more frequent measurements, up to once per second. This process will also result in less sample handling and less contamination for better overall test results.

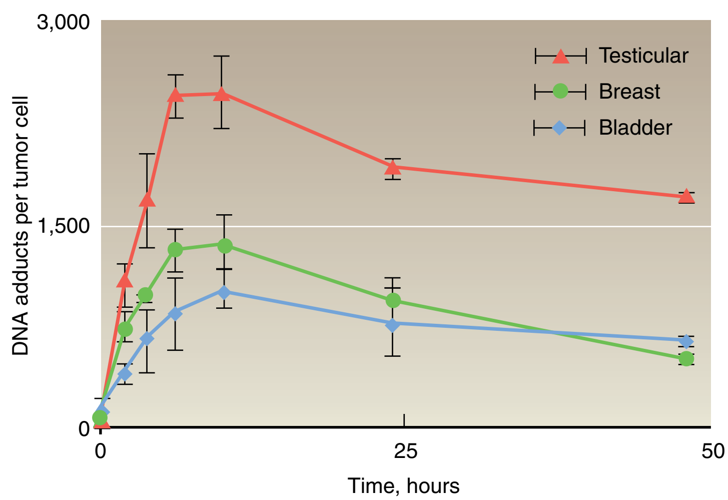
Predicting Patient Response

A collaborative team of Livermore scientists and clinical researchers at the UC Davis Cancer Center is using AMS to test whether DNA damage caused by a single microdose of an anticancer drug will correlate with tumor shrinkage and increased survival times. The ultimate goal is a diagnostic tool that can predict how individual patients will respond to therapy. Says biochemist Paul Henderson, "Genetic screening and microdose testing together would determine the best way to use a particular drug or combination of drugs for a patient."

Chemotherapy agents that damage the DNA of cancer cells are among the most effective compounds for treating cancer. In particular, the platinum-based compounds cisplatin and carboplatin have revolutionized the treatment of many solid tumors. These drugs form damage products, called DNA adducts, that are toxic to rapidly dividing cells. However, a patience's resistance to one of these drugs may cause the drug to produce fewer adducts. Either the patient's tumor may be intrinsically resistant to the drug, or the tumor may shrink for a time and then acquire resistance and stop shrinking. Oxaliplatin, a more recently developed drug, is effective against tumors that are resistant to cisplatin and carboplatin. Both carboplatin and oxaliplatin are compatible with AMS technology because their



Results of a new AMS assay indicate how much carboplatin becomes attached to salmon sperm DNA over 25 hours. These results demonstrated AMS can measure carboplatin–DNA damage, which led to experiments with cancer cells. The next phase is to develop protocols for microdosing experiments with human subjects.



Tests using cultured human cells were performed with oxaliplatin, a chemotherapy drug similar to carboplatin, to determine its effects on the DNA of various types of human cancer cells. Testicular cancer cells (red) are much more responsive to the drug than either breast cancer cells (green) or bladder cancer cells (blue).

carbon-12 atoms can be replaced with carbon-14 atoms.

In an ongoing project funded by Livermore's Laboratory Directed Research and Development Program, the team has developed an AMS-based assay for carboplatin–DNA adducts in cells using purified DNA and several types of cultured human cancer cells. AMS measurements of the kinetics of carboplatin bound to salmon sperm DNA were the first-ever experimental determinations of adduct formation using radioactively labeled carboplatin. Subsequent tests exposed human bladder cancer cells to a microdose of carbon-14-labeled carboplatin. In addition, a variety of human platinum-sensitive and platinum-resistant cancer cells were exposed to oxaliplatin to show that cell-dependent differences in DNA adduct formation and repair are detectable. The highest DNA adduct accumulation was in testicular cancer cells, which may explain why plantinum drugs cure about 95 percent of nonmetastatic testicular cancers. The assay was also tested on mice. This series of tests, undertaken before any experiments with human subjects, indicated that the assay was accurate and safe.

The next step is a clinical study to develop dosing and sample preparation protocols. UC Davis Cancer Center director Ralph deVere White and other UC collaborators will locate five bladder-cancer patients willing to participate as human subjects. The patients will receive a microdose of carbon-14-labeled carboplatin, and the AMS assay will be applied to whole blood, plasma, white blood cells, and biopsy tissue. "We expect to find similar concentrations of DNA adducts in tumor and white blood cells," says Henderson, "which will support the idea that blood cells can be used as a surrogate marker of tumor response." In the future, invasive biopsies could be avoided. Only blood samples would be needed to locate and count carboplatin–DNA adducts.

Fifty bladder- and breast-cancer patients will be recruited for a second study to correlate carboplatin–DNA adduct levels with patient response. AMS analyses will measure the quantity of adducts while computed tomography scans will assess whether tumors have shrunk. Individual responses will be added to a future database built to study pharmacokinetic parameters of these important chemotherapeutic drugs.

Yersinia pestis, or plague (the purple rod-shaped cells), is one of the disease models that will be used if Livermore begins the search for new drugs to fight modified or engineered bioterror agents.

New Drugs for New Diseases

Livermore also hopes to move its AMS expertise in a new direction, applying AMS to the Laboratory's mission in national security, specifically biosecurity. Livermore has a long history of developing methods and devices to detect biological agents that could be used in a potential terrorist attack, and it leads the national BioWatch program. In the near future, Laboratory scientists hope to explore how AMS can be used to develop treatment methods that deal with the aftereffects of a biological attack.

"The nation needs a flexible biodefense strategy," says Dave Rakestraw, chief technologist for the Chemistry, Materials, Earth, and Life Sciences Directorate. "We could stockpile ciprofloxacin [a synthetic antibiotic] to treat anthrax. But anthrax engineered with resistance to ciprofloxacin might appear. We need medical countermeasures that are broadly applicable." A rapid response is critical: the 1918–1919 influenza epidemic killed approximately 25 million people in its first 25 weeks.

The Laboratory's goal is to find broad-spectrum anti-infective drugs that can target agents, such as plague or Hantavirus, which may be modified or engineered. Equally challenging are naturally occurring diseases, such as AIDS, severe acute respiratory syndrome, and perhaps as-yet-unknown pathogens. Rakestraw notes that the National Institutes of Health has spent more than $30 billion on AIDS/HIV research, yet 6,000 people die every day from AIDS. Finding solutions is clearly a challenge.

With expertise in physics, biochemistry, bioinformatics, and genomics, the Laboratory is in an excellent position to identify commonalities among pathogens and ways that they adapt to the people who become infected. Scientists could identify both common and unique factors in the immune response—a critical process for fighting infection—based on research on four particular disease models: *Yersinia pestis* (plague), *Francisella tularensis* (tularemia), *Brucella abortus* (brucellosis), and Hantavirus. All are highly infectious diseases that may infect humans or animals.

Most major pharmaceutical companies have stopped developing anti-infective drugs because the market is too small, and the rapid rate of pathogen evolution shortens the effective lifetime of a drug. Many of these companies also no longer

Accelerator Mass Spectrometry at Livermore

Livermore's Center for Accelerator Mass Spectrometry (CAMS) is home to the most versatile and productive AMS facility in the world. AMS is an exceptionally sensitive technique for measuring concentrations of isotopes in small samples, typically less than 1 milligram, and the relative abundance of isotopes at low levels. It can, for example, find one carbon-14 isotope among a quadrillion other carbon atoms.

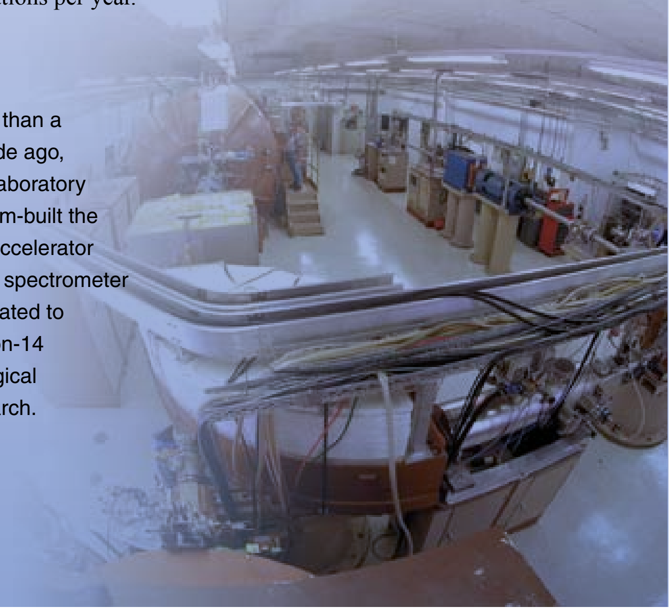
All over the world, AMS is used primarily to count carbon-14 in archaeological and geologic samples for dating purposes. Only at Livermore and a few other sites has AMS been applied to biological research. In 1999, the National Institutes of Health named CAMS as a National Research Resource for AMS. The facility remains the only such research resource for AMS worldwide.

Mass spectrometry has been used since early in the 1900s to study the chemical makeup of substances. The various ions in a sample are sorted by their mass-to-charge ratios in an electric field. The basic principle is that isotopes of different masses move differently in a given electromagnetic field.

In an accelerator mass spectrometer, negative ions made in an ion source are accelerated in a field of hundreds of thousands of volts. The accelerated ions smash through a thin carbon foil or gas that destroys all molecular species. After passing through a high-energy mass spectrometer and various filters, the ions finally slow to a stop in a gas ionization detector. The identity of individual ions can be determined by how the ions slow. For example, carbon-14 slows down more slowly than nitrogen-14, so ions of the same mass can be distinguished from one another.

Once the charges are determined, the detector determines which element each ion belongs to and counts the desired isotope as a ratio of a more abundant isotope. Rare carbon-14 is counted as a ratio of carbon-13, which is quite common. The molecular dissociation process in the accelerator and the ion detection at the end give AMS a sensitivity that is typically a million times greater than that of conventional isotopic detection.

CAMS was established in 1989 to diagnose the fission products of atomic tests and to monitor the spread of nuclear weapons to other countries by detecting telltale radioisotopes in air, water, and soil samples. In addition, plans were to develop isotopic tracers for studying climate and geologic records and to use AMS technology for biomedicine applications. Today, CAMS performs these services, and many others, 24 hours a day, 7 days a week for Livermore researchers and their collaborators as well as for others on a fee-for-service basis. The facility performs more than 25,000 AMS measurement operations per year.



More than a decade ago, the Laboratory custom-built the first accelerator mass spectrometer dedicated to carbon-14 biological research.

develop new anti-infectives because existing chemical libraries have run out of the natural products on which anti-infective drugs are based. Most such drugs come from naturally occurring microbes in soils and plants.

Livermore has proposed a collaborative effort with Trius Therapeutics of San Diego, California, a firm that specializes in developing antibacterial drugs to treat infections caused by antibiotic-resistant bacteria. Says John Finn, chief scientific officer at Trius, "We would first identify a pool of possible drug candidates based on an analysis of molecular structures that fit the disease models. Microdosing experiments with AMS would then give us the metabolic data we need to narrow the field. Highly precise data on pharmacokinetics is critical." Says Rakestraw, "Together, we have the expertise to develop entirely new anti-infective drugs. A new chemical library would be based on natural products from oceans and other water sources."

AMS has repeatedly proved its value in biological research. Applying this expertise to the challenge of countering bioterrorism would give the Laboratory a unique capability in helping to protect our nation. Says Turteltaub, "With AMS, we can address a host of health problems that cannot be solved otherwise."

—Katie Walter

Key Words: accelerator mass spectrometry (AMS), ADME (absorption, distribution, metabolism, excretion), biosecurity, cancer, Center for Accelerator Mass Spectrometry (CAMS), chemotherapy, microdosing, pharmacokinetics, University of California (UC) Davis Cancer Center.

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